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First report and morphological, molecular characterization of *Xiphinema chambersi* Thorne, 1939 (Nematoda, Longidoridae) in Canada

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Abstract

A *Xiphinema* species, new to Canada was recovered from rhizosphere of oak trees in Ontario, Canada. The identity was confirmed with morphological and molecular methods as *X. chambersi* Thorne, 1939. Female bodies are 2.1–2.4 mm long; odontostyle lengths are 110–118 µm; tail 110–177 µm long, arcuate, elongate-conoid, with hyline region 22 - 43 µm long. Vagina directed about 30 degrees posteriorly. Reproductive system is monodelphic with ovary reflexed anteriorly, vulva opening at 23–26% of the body. Males were not found. The 18S and ITS1 sequences of this population had 3–4 bp differences (99% identity) and 30 bp differences (97% identity) from two Arkansas populations respectively. The nematode population had three juvenile stages. Some variations of the morphometrics were observed comparing with the other populations. This is the first report of *X. chambersi* in Canada.

Keywords

Xiphinema, dagger nematode, diagnostic, Canada, rDNA sequencing

Introduction

Xiphinema chambersi Thorne, 1939 was described from specimens from Virginia, USA. A more complete description of this species was added by Cohn and Sher (1972) based on the lectotypes. In USA, *X. chambersi* has been reported in 16 states and is widely

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distributed (Cohn and Sher 1972, Robbins and Brown 1991, Ye 2002, Lamberti et al. 2002). It was also reported in Japan (Shishida 1983) and Korea (Cho et al. 1992). Males were only reported in Arkansas (Ye 2002). The Japan (Shishida 1983) and Florida (Lamberti et al. 2002) populations have been reported having 3 juvenile stages, the other reported populations have not been specified on the number of juvenile stages. Although Thorne (1939) described and illustrated a male, no males were seen on the syntype slides. Only 2 males were found in Arkansas (Ye 2002). This species has been reported to cause damage on sweet gum (*Liquidambar styraciflua*) in Georgia (Ruehle 1971) and strawberry (Perry 1958). During a survey of nematodes of grasslands in Ontario, a population of *Xiphinema* was discovered. It was identified as *X. chambersi*. The objective of this paper is to report and characterize *X. chambersi* in Ontario, Canada with morphological and molecular methods.

Materials and methods

Nematodes. Soil samples were collected with a soil probe from the rhizosphere of oak trees (*Quercus rubra* L.) at the Turkey Point Provincial Park of Ontario (42°42.460'N, 80°20.375'W), Canada in 2009. Nematodes were extracted with the modified pan method (Townshend 1963), fixed in TAF, processed to glycerine and mounted on slides (Hooper 1970) for compound microscopic studies. A portion of the nematodes were frozen at -20°C in water for molecular studies.

Microscopic observation. Specimens were examined using Leica DM5500 B compound microscope using differential interference contrast and pictures were taken with Leica DFC 420 digital camera. The observed characters of the adults were compared with those of the specimens described by Thorne (1939) and the description by Cohn and Sher (1972). Measurements were made using a Leica micro application system on the images, and dimensions are expressed in a formula suggested by de Man (1880). Drawings were aided using a drawing tube.

SEM. Nematodes were first fixed in TAF (7% formalin, 2% triethanolamine, 91% distilled water), and then transferred to Seinhorst solution 1 (1% glycerol; 4% formalin: 95% distilled water) in a foam capsule. The nematodes then were processed through a serial alcohol dehydration by placing in the foam capsule in succession into 40, 60, 70, 90, and 100% ethanol each for 24 h. the foam capsule with the nematodes was placed into a Polarum E3100 Jumbo II CPD, where critical point drying of the nematodes was accomplished utilizing carbon dioxide. The nematodes were then glued on pins using the wood glue as adhesive. The pins with nematodes were then placed in an Emitech K550X Sputter Coater and coated for 1 min with gold-palladium. Nematodes were observed using a Philips XL 30 Scanning Electron Microscope.

Molecular study. Two populations of *X. chambersi* from Arkansas were included for the molecular comparisons. Both ITS and 18S genes of the Ontario population and Arkansas populations were PCR amplified and sequenced. Primers were the same as described in Ye et al. (2004) and Neilson et al. (2004). The 25 µl PCR contained

12.5 µl 2X GoTaq DNA polymerase mix (Promega Corporation, Madison, WI 53711, USA), 1 µl each of 0.4-µM forward and reverse primers and 1 µl of DNA template. The thermal cycling program was as follows: denaturation at 95°C for 6 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 1 min. A final extension was performed at 72°C for 10 min. PCR products were cleaned by Montage™ PCR Centrifugal Filter Devices (Billerica, MA, USA). PCR primers were used for direct sequencing by dideoxynucleotide chain termination using an ABI PRISM BigDye terminator cycle sequencing ready reaction kit (Applied Biosystems) in an Applied Biosystems 377 automated sequencer (Applied Biosystems) by Eurofins MWG Operon (Huntsville, AL35805, USA). The sequence was deposited in Genbank. The sequence was compared with other nematode species stored at the GenBank using the BLAST homology search program. The closest sequences were selected in the phylogenetic analysis. DNA sequences were aligned by Clustal W (http://workbench.sdsc.edu, Bioinformatics and Computational Biology group, Dept. Bioengineering, UC San Diego, CA). Maximal-parsmony (MP) analysis (Saitou and Nei 1987) was conducted on both ITS and 18S. Sites with missing data or gaps were treated as missing characters for the analysis. The robustness of the MP tree was tested using the bootstrap method (Felsenstein 1985) based on 2,000 replicates.

Results and discussion

The specimens are deposited in the Canadian National Collection of Nematode. Morphometrics data of the females and 3 stages of juveniles are in Table 1.

Morphological characterization

The main characters of *X. chambersi* from Ontario, Canada match well with the lectotype described by Cohn and Sher (1972) and Arkansas populations (Ye 2002) regarding to the body shape, size, the tail shape, hyline tail length, the odontostyle length, and the female gonad. It has only 3 juvenile stages, and no male was found.

Female. Body is C- to L- shaped when relaxed, tapering off gradually at both ends, more so posterior. Lip region is slightly setoff from the rest of body by a faint depression. Amphid aperture is about four-fifth of the width of lip region. Reproductive system is monodelphic, opistodelphic with ovary reflexed. Vagina directed about 30 degrees angle posteriorly, lips thick. Intestine is transparent, pre-rectum is visible, and rectum is 45 µm long. Tail is 132 µm long, arcuate, elongate-conoid with 3 pairs of caudal pores, terminating in a cylindroid nonprotoplasmic tip with hyline region of 37.4 µm long.

Most of the morphometirc measurements of the females agree with those described by Cohn and Sher (1972) and Ye (2002) in the USA and Japan, some morphometrics variations have been observed: The averaged body length of the female of the population in Ontario, Canada is 2.2 ± 0.1 (2.1–2.4) mm is smaller than that of the populations

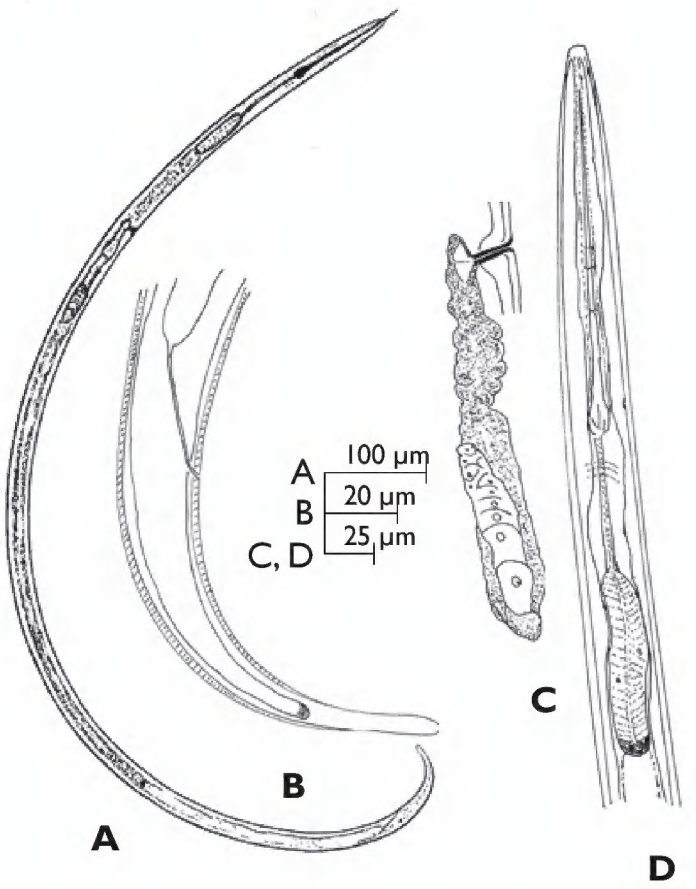

Figure 1. Drawings of female of *Xiphinema chambersi* from Ontario, Canada, **A** entire body **B** tail region **C** gonad **D** pharynx.

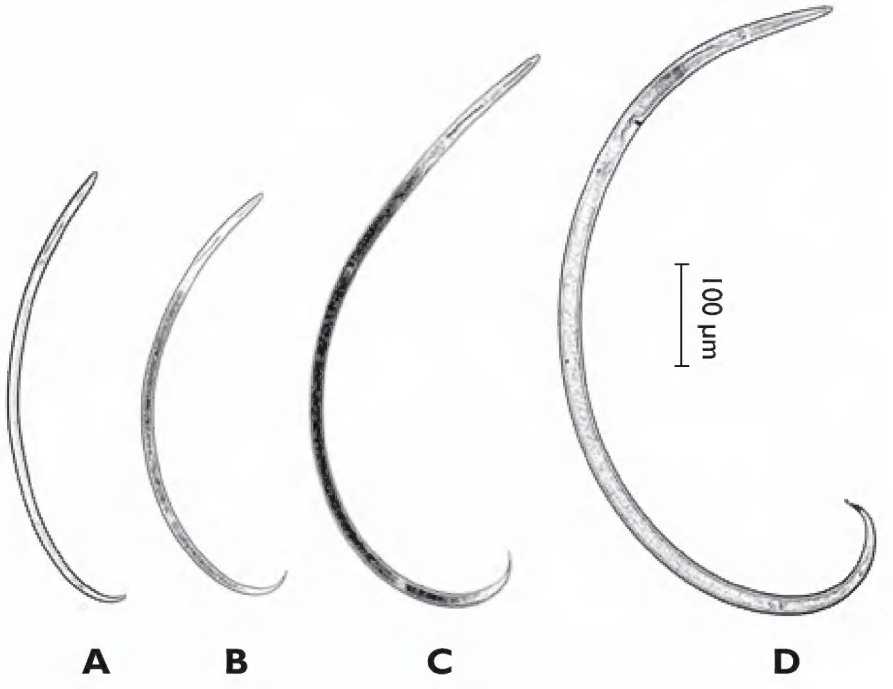

Figure 2. Juveniles and female of *Xiphinema chambersi* from Ontario, Canada, **A** first stage juvenile **B** second stage juvenile **C** third stage juvenile **D** female.

from Iowa, USA, the Florida population averaged 2.5±0.15 (2.4–2.7) µm (Lamberti et al. 2002) the Arkansas population averaged 2.5 (2.1–2.8) µm (Ye 2002) and the Iowa population 2.4 (2.2–2.5) µm (Cohn and Sher 1972); but is larger than that of the population in Japan averaged 1.9 (1.8–2.0) µm (Shishida 1983). The odontostyle lengths of females from these populations are similar. The hyline tail length of the Ontario population averaged 37.4 ± 6.1 (22.0–43.4) µm is much longer than that of the population

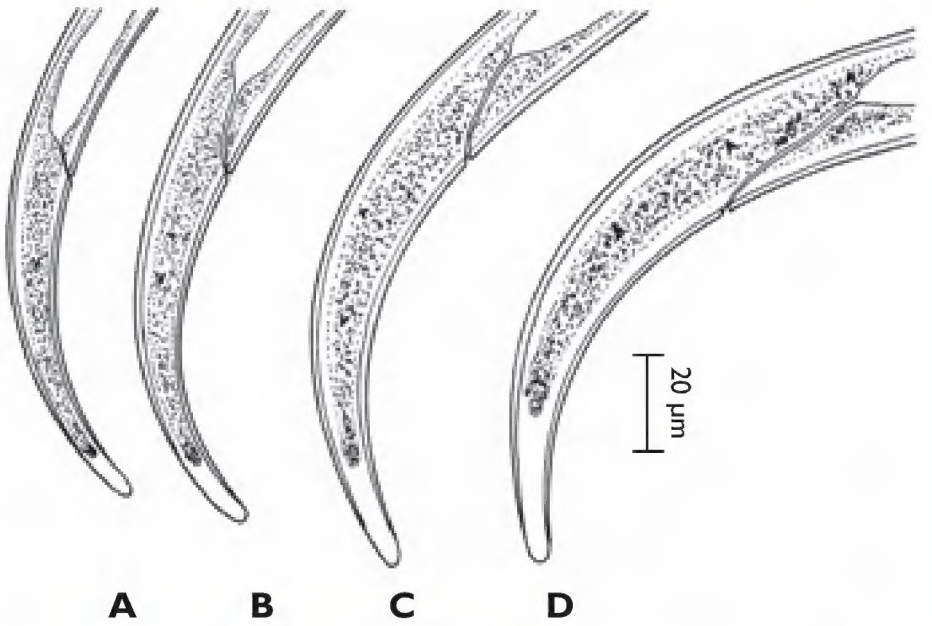

Figure 3. Comparison of tails of *Xiphinema chambersi* from Ontario, Canada, **A** first stage juvenile **B** second stage juvenile **C** third stage juvenile **D** female.

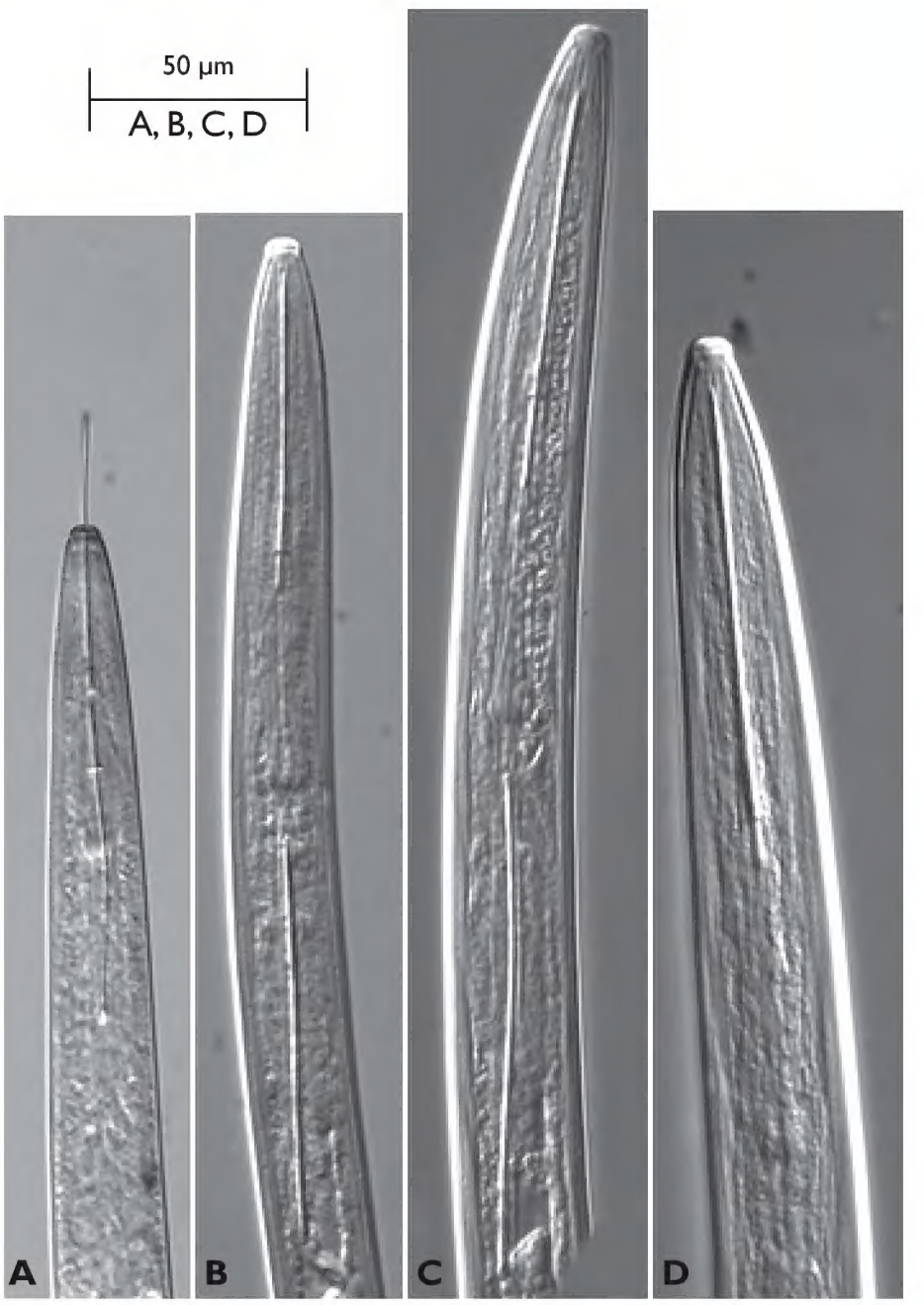

Figure 4. Comparison of pharyngeal regions of different stage of juveniles and female of *Xiphinema chambersi* from Ontario, Canada, **A** first stage juvenile **B** second stage juvenile **C** third stage juvenile **D** female.

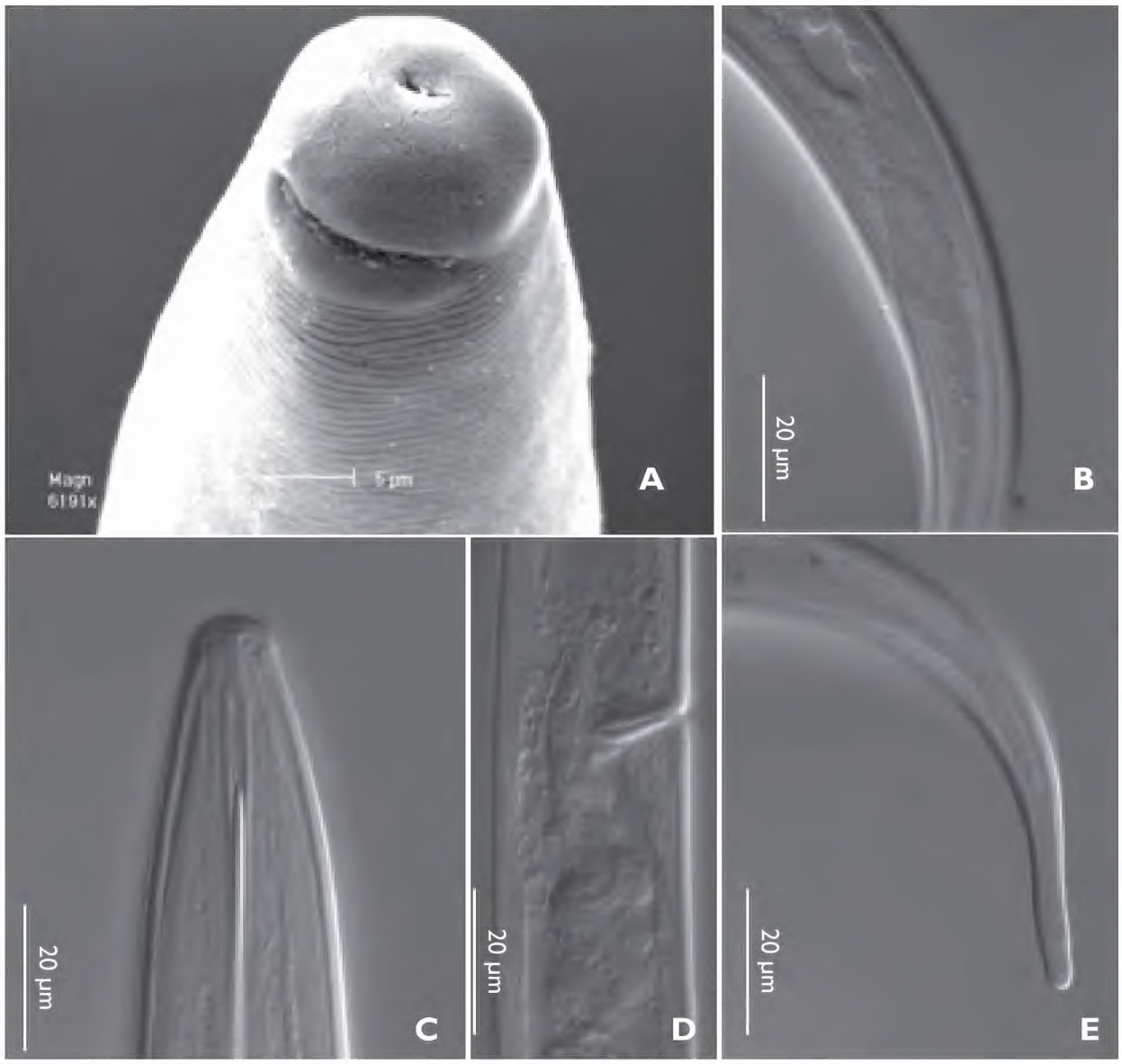

Figure 5. Micrographs of *Xiphinema chambersi* from Ontario, Canada, **A** SEM image of lip region of female **B** anus region **C** head **D** vulva **E** tail.

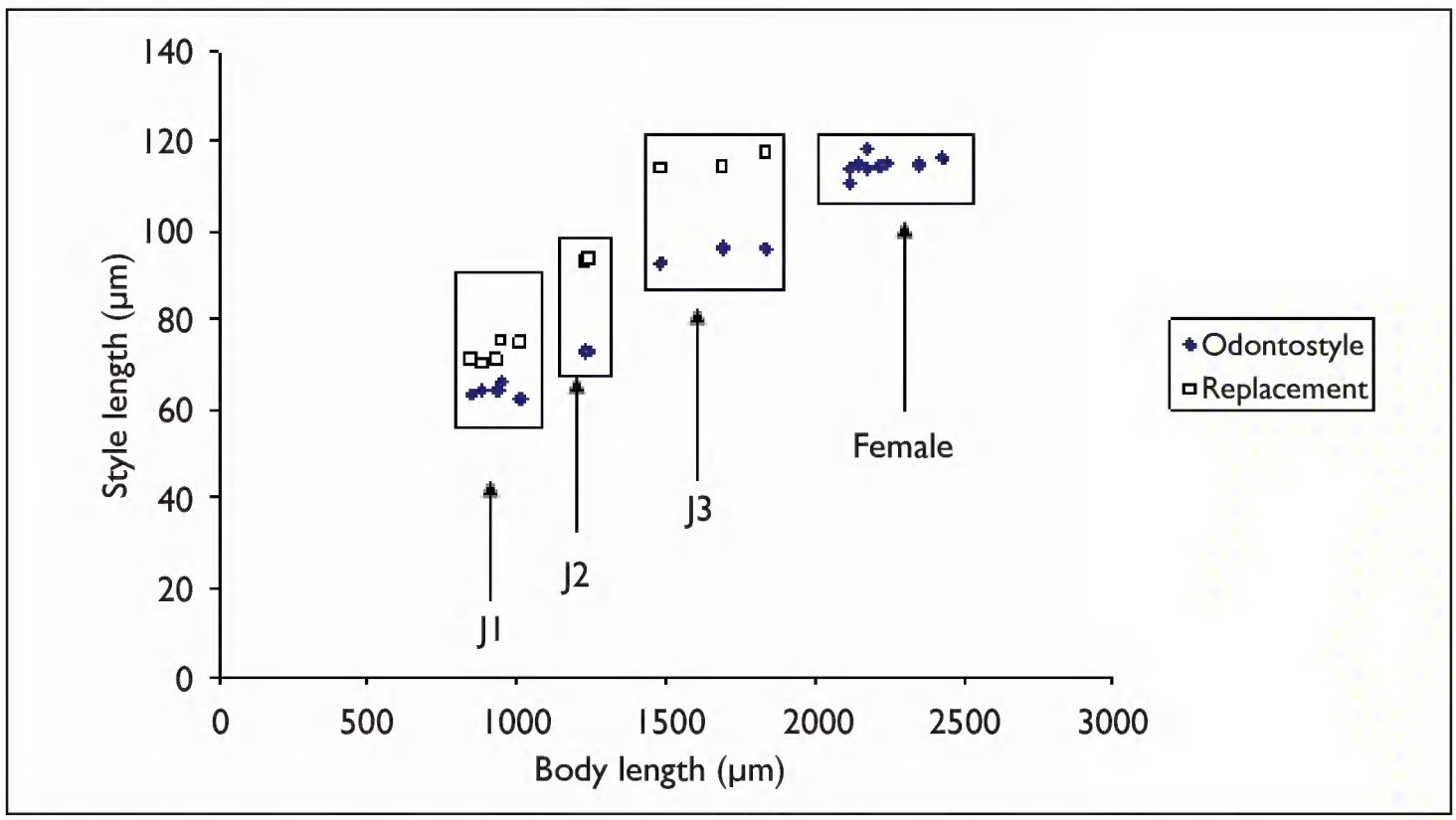

Figure 6. Scatter diagram plotting body, odontostyle, and its replacement of individual juveniles and females of *X. chambersi* from Ontario, Canada.

Table 1. Morphometric data of *X. chambersi* from Canada

Character	J1	J2	J3	Female
n	5	2	3	9
L (mm)	0.9±0.1	1.2±0.1	1.7±0.2	2.22±0.1
	(0.8–1.0)	(1.2–1.2)	(1.5–1.8)	(2.1–2.4)
Total stylet	103±2.7	122.1±0.6	152.3±3	180.5±3.3
	(98.5–105.8)	(121.5–122.6)	(148.3–155.8)	(173.0–185.1)
Odontostyle	64.1±1.2	72.9±0.1	94.9±1.5	114.7±1.9
	(62.4–66.1)	(72.8–73.0)	(92.8–96.0)	(110.5–118.1)
Odontophore	38.9±2.6	49.1±0.5	57.4±1.8	65.9±2.4
	(35.1–43.1)	(48.7–49.6)	(55.5–59.9)	(62.5–70.2)
Odontostyle replacement	72.5±2.2	93.4±0.4	115.3±14.3	-
	70.1–75.4	92.99–93.9	113.9–117.3	-
a	40.4±3.8	48.1±1.6	50.5±2	51.9±2.5
	(32.7–43.3)	(46.5–49.8)	(47.7–52.3)	(47.7–55.7)
b	3.7±0.3	3.8±0.2	4.4±0.2	5.8±0.3
	(3.2–4.1)	(3.7–4.0)	(4.2–4.7)	(5.4–6.2)
c	11.4±0.4	12±0.1	14.2±1.1	17±1.9
	(10.7–11.9)	(11.9–12.1)	(12.6–15.2)	(12.5–19.7)
c'	5.7±0.5	5.8±0.3	5.3±0.3	5.3±0.7
	(5.1–6.6)	(5.5–6.1)	(5.1–5.7)	(4.4–7.0)
V	-	-	-	24.4±0.9
	-	-	-	(23.1–26.4)
Lip region width	7.9±0.2	8.8±0.1	9.9±0.1	10.9±0.6
	(7.6–8.1)	(8.7–8.9)	(9.7–10.0)	(10.0–12.0)
Lip region height	2.8±0.2	4.3±0	4.2±0.1	5.3±0.8
	(2.6–3.0)	(4.3–4.3)	(4.1–4.4)	(4.5–7.2)
Guiding ring from anterior end	56.7±1.8	72.9±0.6	88.1±0.2	110±3.3
	(53.2–57.9)	(72.4–73.6)	(87.8–88.3)	(105.4–115.0)
Guiding sheath	19.9±6.1	10.3±0.3	6.3±1.9	10.2±3.7
	(8.6–25.2)	(10.1–10.6)	(4.4–8.9)	(4.4–15.7)
Flanges width	8.1±0.4	8.6±0.3	10.3±0.4	10.1±0.6
	(7.4–8.4)	(8.3–8.8)	(9.8–10.8)	(9.2–11.0)
Body width at midbody	23.1±3.2	25.8±1.1	33±1.9	42.9±2.4
	(20.1–28.9)	(24.7–26.8)	(31.1–35.7)	(39.9–47.8)
Pharyngeal bulb length	63.8±1.8	74.1±0.9	89.3±0.9	95.9±5.4
	(61.4–65.9)	(73.2–75.1)	(88.5–90.2)	(86.4–103.0)
Pharyngeal bulb width	12.3±0.9	12.9±1	15.6±1.5	17.2±1.4
	(11.0–13.2)	(12.0–14.0)	(14.2–17.7)	(15.7–20.0)
Pharynx	252.3±6.8	323.2±12.8	376.7±14.7	384.9±13.5
	(245.6–264.2)	(310.4–336.0)	(356.9–392.2)	(359.4–403.0)
G1	-	-	-	16.5±1.7
	-	-	-	(13.9–21.0)

Character	J1	J2	J3	Female
n	5	2	3	9
G1%	-	-	-	0.7±0.1
	-	-	-	(0.6–0.9)
G2	-	-	-	195.7±37.2
	-	-	-	(154.6–289.2)
G2%	-	-	-	8.8±1.8
	-	-	-	(7.2–13.3)
Prerectum	111.1±19.9	163.4±27.5	184.3±20.2	278.7±67
	(72.3–128.4)	(135.9–190.8)	(155.8–199.0)	(215.0–413.7)
Rectum	14.7±0.9	25.5±0.9	35.4±2	45.2±5.8
	(13.4–15.8)	(24.5–26.5)	(33.4–37.4)	(37.5–56.9)
Tail length	81±2.8	102.9±1.6	117.6±2.3	132.1±17.6
	(76.3–84.6)	(101.2–104.5)	(115.1–120.7)	(110.2–177.3)
Body width at anus	14.4±1.3	17.8±1.3	22.1±1.2	24.9±1.6
	(12.4–16.1)	(16.6–19.1)	(20.5–23.1)	(23.4–28.9)
Hyaline tail tip	11.9±0.7	18.4±2.2	28.4±1.8	37.4±6.1
	(11.0–13.1)	(16.2–20.5)	(26.0–30.2)	(22.0–43.4)
H%	14.7±0.6	17.9±2.4	24.1±1.1	28.5±4.8
	(13.9–15.5)	(15.5–20.3)	(22.6–25.0)	(19.9–34.4)

Note: unless specified in the table, the unit for the measurements is μm.

from Florida averaged 20.4 ±2.1 (17.6–23) μm, and Arkansas's averaged 29.7 (15.0–35.0), is shorter than that of the population from Japan averaged 43.7 (38–47) μm.

Juvenile. The presence of three juvenile stages was determined. The body of the first stage juvenile ventrally curved slightly when heat relaxed, the anterior end of the replacement odontostyle almost adjacent to the posterior end of the functional odontostyle, the tail has one pair of caudal pores. The body of the second stage juvenile is curved slightly more than the first stage juvenile, the anterior end of the replacement odontostyle is behind the posterior end of the functional odontostyle. The tail has 2 pairs of caudal pores. The body of the third stage juvenile is ventrally curved slightly more than the second stage juvenile, especially in the tail region, the anterior end of the replacement odontostyle is more posterior to the end of the functional odontostyle. The tail has three pairs of caudal pores. Fig. 6 revealed *X. chambersi* from Canada only has 3 juvenile stages.

Male: not found

Molecular characterization

DNA Sequence: 2610 bp ribosome DNA segment consisting of the near-full-length 18S ribosomal RNA gene, the internal transcribed spacer 1 and partial 5.8S ribosomal

RNA gene was PCR amplified and sequenced for Ontario population of *X. chambersi* (genBank accession number HM138503). The 18S sequence of this population had only 3–4 bp differences (99% identity) from two Arkansas populations sequenced (AY283174 for population Xiph-41 and HM191718 for population Xiph-61). ITS1 region between Ontario population and Arkansas population Xiph-61 had 30 bp differences (97% identity, 1029 total characters) and 4 gaps. Phylogenetic trees based on the 18S and ITS of rDNA agreed with the results from previous studies (Ye et al. 2004, Neilson et al., 2004) (trees not shown). Both trees revealed the closest relationships being in the same highly supported monophyletic clade for the Ontario population and the Arkansas populations of *X. chambersi*. Due to the short branch length difference in phylogenetic trees and lack of sufficient morphological differences among *X. chambersi* populations studied, the DNA sequence differences on 18S and ITS were considered as intraspecies variation when many *Xiphinema* species and populations were examined in the phylogenetic trees.

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References

Cohn E, Sher SA (1972) A contribution to the taxonomy of the Genus *Xiphinema* Cobb, 1913. Journal of Nematology 4: 36–65.

Cho YE, Choi YS, Cho MR (1992) Nematodes associated with forest trees in Korea. III. A new species of *Xiphinemella* Loos, 1950 and four unrecorded species of *Xiphinema* Cobb, 1913. Korean Journal of Applied Entomology 31: 416–426.

Hasagawa M, Kishino H, Yano TA (1985) Dating of the human ape splitting by a molecular clock of mitochondrial-DNA. Journal of Molecular Evolution 22: 160–174.

Hooper DJ (1970) Handling, fixing, staining and mounting nematodes. In: Southey JF (⊠d) Laboratory methods for work with plant and soil nematodes. London, 59–80

Lamberti F, Luca FDe, Molinari S, Duncan LW, Agostineli A, Coiro ML, Dunn D, Radicci V (2002) *Xiphinema chambersi* and *Xiphinema naturale* sp. n., two monodelphic Longidorids (Nematoda, Dorylaimida) from Florida. Nematologia Mediterranea 25: 55–61.

Neilson R, Ye W, Oliveira CMG, Hübschen J, Robbins RT, Brown DJF, Szalanski AL (2004) Phylogenetic relationships of selected species of Longidoridae (Nematoda: Longidoridae) from North America inferred from 18S rDNA gene sequence data. Helminthologia 41: 209–215.

Perry VG (1958) Parasitism of two species of dagger nematodes (*Xiphinema americanum* and *X. chambersi*) to strawberry. Phytopathology 48: 420–423.

Robbins RT, Brown DJF (1991) Comments on the taxonomy, occurrence and distribution of Longidoridae (Nematoda) in North America. Nematologica 37: 395–419.

Ruehle JL (1968) Plant-parasitic nematodes associated with southern hardwood and coniferous forest trees. Plant Disease Report 52: 837–839.

Saitou N, Nei M (1987) The neighbor-joining method, a new method for reconstructing phylogenetic trees. Molecular Biology Evolution. 4: 406–425.

Shishida Y (1983) Studies on nematodes parasitic on woody plants 2. Genus Xiphinema Cobb, 1913. Japanese Journal of Nematology 12: 1–14.

Thorne G (1939) A monograph of the nematodes of the super-family Dorylaimoidea. Capita Zoologica 8: 1–261.

Townshend JL (1963) A modification and evaluation of the apparatus for the Oostenbrink direct cottonwool filter extraction method. Nematologica 9: 106–110.

Ye W (2002) Morphological and molecular taxonomy of *Longidorus* and *Xiphinema* (Nematoda: Longidoridae) occurring in Arkansas, USA. PhD dissertation, University of Arkansas, 380 pp.

Ye W, Szalanski AL, Robbins RT (2004) Phylogenetic relationships and genetic variation in *Longidorus* and *Xiphinema* species (Nematoda: Longidoridae) using ITS1 sequences of nuclear ribosomal DNA. Journal of Nematology 36: 14–19.